**Supplementary Materials and Methods**

**Cell culture**

All cell lines were maintained in RPMI1640 medium supplemented with 10% FBS and 1% antibiotic/antimycotic in an incubator with 5% CO2 at 37°C. HEK293 FT cells were cultured in DMEM with 10% FBS and 50 mg/ml Gibco Geneticin.

**RNA interference**

Cells were treated with either ON-TARGETplus SMARTpool AXL siRNA and ON-TARGETplus non-targeting control pool siRNAs (Horizon Discovery) using Lipofectamine RNAiMAX (Thermo Fisher) for gene silencing as per manufacturer’s protocol.

**Immunohistochemistry**

Lung tumor tissue microarrays used have been previously described (1). Specimens were collected with patient’s consent through an institutionally approved protocol and in accordance with federal guidelines. 5 µm sections were stained using a Ventana Discovery XT automated system (Ventana Medical Systems, Tucson, AZ) as per manufacturer's protocol. Briefly, slides were deparaffinized on the automated system with EZ Prep solution (Ventana) followed by heat-induced antigen retrieval in Cell Conditioning 1 (Ventana). Slides were then incubated for 60 min with an AXL antibody (Cell Signaling, 8661), followed by the Ventana OmniMap Anti-Rabbit Secondary Antibody for 16 min and visualized using the Ventana ChromoMap kit. Slides were then counterstained with Hematoxylin, dehydrated and hard-mounted. Evaluation of immunohistochemical expression of AXL was performed using a grading system where the staining intensity was graded as 0 = none, 1 = weak, 2 = moderate, and 3 = strong.

**Immunofluorescence**

Cells were plated at ~ 40 x 104 per well in eight-well chamber slides, grown on the slides for 24-48 hours, exposed to appropriate treatments, fixed with 4% paraformaldehyde, and permeabilized with 0.5% Triton X-100. Following blocking of non-specific binding by incubation with 1.5% bovine serum albumin (BSA) at room temperature for 30 min, primary antibody (anti-FLAG antibody, Proteintech, 20543-1-AP), diluted in 0.15% BSA, was added overnight at 4°C. The next day, slides were incubated with a goat anti-rabbit Alexa Fluor 488 (A-11008, Thermo Fisher Scientific) secondary antibody for an hour at room temperature before mounting with ProLong Gold antifade mounting medium with DAPI (Thermo Fisher Scientific). Images were acquired on a confocal microscope (Leica SP8) at 20X.

**BioID sample preparation and data analysis**

Samples for BioID proteomics analysis were prepared using methods described in (2) with a few modifications. H1299- pSTV6-C-BirA\*-FLAG-AXL cells were cultured in DMEM medium supplemented with 10% FBS and 1% antibiotic-antimycotic and induced with 1µg/ml doxycycline (Dox) and 40 μM biotin for 24 hours once they reached ~75% confluence. Cells were washed and collected in cold PBS, pelleted and frozen until time of sample processing. Pellets were lysed in 1 ml of RIPA lysis buffer (Thermo Fisher Scientific) with 1 mM PMSF and 1x Protease Inhibitor cocktail solution (GenDepot) and sonicated as described before (2). Samples were incubated with 250 U of Turbonuclease (Biovision Inc.) and 10 μg of RNase A (15 min at 4°C) and then 30 μl of pre-washed NeutrAvidin beads (Thermo Fisher Scientific) were added to sample supernatants. After 4 hours of rotation at 4°C, the beads were washed once with SDS wash buffer (25 mM Tris-HCL, pH 7.4, 2% SDS), twice with RIPA buffer, once with TNNE buffer (25 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 0.1% NP40), and three times with 50 mM ammonium bicarbonate pH 8.0 (ABC buffer). 20 µL of SDS-PAGE running buffer, 2 µL of reducing agent, and 10 µL of loading buffer were then added to the beads. The samples were heated at 95°C for 5 minutes to denature and elute the proteins. Sample supernatants were removed from beads and loaded onto a 10% Criterion SDS-PAGE gel. The SDS-PAGE was run for 5-7 minutes at 125 V to capture the proteins in the gel for buffer exchange and in-gel digestion. Gel was removed from cassette, rinsed, stained overnight with InstaBlue dye, de-stained in 10% methanol/5% acetic acid and imaged. All bands were cut and placed in microcentrifuge tubes, then diced into 1 mm squares. An in-gel digest was performed, including de-staining, reduction, alkylation, and digestion with 200 ng of trypsin. After overnight incubation at 37°C, another 200 ng trypsin was added and digestion continued for 2 more hours. After digestion, peptides were extracted from gel bands using 50% acetonitrile, 0.1% TFA. Samples were dried in a vacuum centrifuge and resuspended in aqueous 1% Trifluoroacetic acid (TFA), 2% acetonitrile with Peptide Retention Time Calibrator or PRTC peptide standards (Thermo) added. Samples were desalted and cleaned using Millipore C18 ZipTips. The clean peptide elution was dried down, and samples were resuspended in 20 µL of 2% acetonitrile, 0.1% formic acid and transferred to autosampler vials for subsequent LC-MS/MS analysis.

Raw files were analyzed, peptides identified using Proteome Discoverer (2.2.0.388) and summarized using Scaffold (4.8.7). Enzyme specificity was set to full trypsin digestion, allowing up to 2 missed cleavages. The mass measurement tolerance was set to 20 ppm for MS and 0.05 Da for MS/MS. Carbamidomethylation of cysteine and oxidation of methionine were set as variable modifications.

To identify proteins that were enriched above the background contaminants detected in the negative control samples and nominate proximity partners of AXL, Automated Processing of SAINT Templated Layouts (APOSTL) software was used as described (3). These proteins were then were queried for known functional and physical interactions using STRING (4) network analysis (high confidence of 0.7). The resulting undirected network was imported into Cytoscape (v. 3.5.1) for visualization (5). KEGG and Gene Ontology (GO) enrichment analyses were also performed on selected proteins using APOSTL.

**Immunoblotting**

Cells were washed once in cold PBS, scraped and lysed in NETN buffer (50 mM Tris, pH 8, 150 mM NaCl, 1 mM EDTA, 0.5% NP-40) containing Xpert protease and phosphatase inhibitor cocktails (GenDepot). Whole cell lysates were resolved by SDS-PAGE and transferred to nitrocellulose membranes. The membranes were blocked in 5% skim milk/PBST, and then incubated overnight with primary antibodies at 4°C followed by incubation with HRP-conjugated anti-rabbit/anti-mouse secondary antibodies (GE Healthcare) for 1 hour at room temperature. Signals were visualized using the SuperSignal West Pico Plus Chemiluminescent Substrate (Thermo Fisher Scientific). Primary antibodies were obtained from Cell Signaling (Danvers, MA): pAXL Y702 (5724), AXL (8661), pERK (4370), ERK (9102), pAKT T308 (13038), pAKT S473 (4060), AKT (9272) and Sigma: β-actin (A5441).

**Phosphoproteomics sample preparation and mass spectrometry**

Phosphoproteomics samples were prepared using the PTMScan Kit (Cell Signaling) as per the manufacturer’s protocol. Briefly, collected cell pellets were first lysed in urea buffer and extracted proteins (~ 20 mg) were then reduced by dithiothreitol, alkylated by iodoacetamide, and digested by trypsin. Peptide purification was performed using Sep-Pak C18 columns (Waters) and then lyophilized. Peptides were dissolved in Immunoaffinity buffer and enriched for phosphotyrosine peptides using the antibody beads (PTMScan Phospho-Tyrosine Rabbit mAb (P-Tyr-1000) Kit, Cell Signaling #8803). Samples were then eluted, concentrated to 20 μL by vacuum centrifugation (Speedvac) and analyzed with LC-MS/MS for label-free quantitation.

The flow through from the immunoprecipitation of phosphotyrosine peptides was saved and used for global phosphoproteomics (pSTY). An aliquot of peptides from each sample (corresponding to the digest of 200 µg of total protein) was labeled using TMT 10-plex reagents following the manufacturer’s recommendation (TMT10plex™ Isobaric Label Reagent Set, Thermo Fisher Scientific). Label efficiency was confirmed by LC-MS/MS; all samples showed more than 98% labelling efficiency by spectral counting. Samples were combined, lyophilized overnight and re-dissolved with 250 µl of aqueous 20 mM of ammonium formate (pH 10.0). The high pH reversed phase separation was performed on a XBridge 4.6 mm x 100 mm column packed with BEH C18 resin with 3.5 µm particle size and 130Å pore size (Waters). The eluted fractions were split; 5% of the total peptides were concatenated into 24 fractions for protein expression, and the remaining 95% of the peptides were concatenated into 12 fractions for phosphopeptide enrichment. Peptides were lyophilized and pSTY peptides enriched using immobilized metal affinity chromatography or IMAC magnetic beads (Cell Signaling Technology) on a KingFisher robot (ThermoFisher). The phosphopeptides were eluted with elution buffer (aqueous 50% ACN, 2.5% Ammonia) and the volume was reduced to 20 µl via vacuum centrifugation.

A nanoflow ultra high performance liquid chromatograph (RSLCnano, Thermo, Sunnyvale, CA) coupled to a quadrupole-orbitrap hybrid mass spectrometer (Q Exactive Plus, Thermo, San Jose, CA) was used for tandem mass spectrometry peptide sequencing experiments. The sample was first loaded onto a pre-column (2 cm x 100 µm ID packed with C18 reversed-phase resin, 5µm particle size, 100Å pore size) and washed for 8 minutes with aqueous 2% acetonitrile and 0.1% formic acid. The trapped peptides were eluted onto the analytical column, (C18, 75 µm ID x 25 cm, 2 µm particle size, 100Å pore size, Thermo, Sunnyvale, CA). The 90-minute gradient was programmed as: 95% solvent A (aqueous 2% acetonitrile + 0.1% formic acid) for 8 minutes, solvent B (aqueous 90% acetonitrile + 0.1% formic acid) from 5% to 38.5% in 60 minutes, then solvent B from 50% to 90% B in 7 minutes and held at 90% for 5 minutes, followed by solvent B from 90% to 5% in 1 minute and re-equilibrate for 10 minutes. The flow rate on the analytical column was 300 nl/min. Twenty tandem mass spectra were collected in a data-dependent manner following each survey scan. The resolution settings were 60,000 and 45,000 for MS and MS/MS, respectively. The isolation window was 0.8 Th with 0.2 offset to capture the M and M+1 isotopes of the peptide precursor.

**Phosphoproteomics data analysis**

Phosphoproteomics data were searched against human entries in the UniProt database using MaxQuant 1.5.2.8 (6). The m/z tolerance for MS/MS scans was set to 20 ppm for MS and 0.05 Da for MS/MS Carbamidomethylation of cysteine was searched as a fixed modification; variable modifications were set to oxidation of methionine, acetylation of the protein N-terminus and phosphorylation of serine, threonine and tyrosine. The false discovery rate (FDR) was set to 0.05.

MaxQuant data were normalized with IRON (Iterative Rank-Order Normalization) against the median sample within each dataset (7). All abundances were log2-transformed prior to all further analyses and technical replicates were averaged. Log2 ratios between treatment groups and DMSO were calculated by subtracting the averaged log2 ratios of the biological replicates of each group. Additionally, t-tests (two-tailed, unequal variance) were calculated for each of the desired two-group comparisons. Differential expression (DE) between conditions was determined using 1.5-fold change and p-value < 0.05 cutoffs. Phosphopeptides from the global and tyrosine phosphoproteomics, which had reported abundances for at least two biological replicates and were DE in at least one condition were combined, assigned to five broad pattern categories as shown in the bottom of Figure 2C, and assigned a consensus sign based on the direction of change at 0.5h. A consensus sign for each gene was determined by taking the majority sign, excluding the ones with equal numbers of observed (+) and (-) directions. Consensus sign lists were generated for both the 1.5-fold, p < 0.05 DE (relaxed) analysis, as well as for a stricter 4-fold, p < 0.001 (strict) cutoff. Strict consistent signs were then filtered to remove genes absent in the relaxed consensus sign list and genes exhibiting opposite consensus signs between relaxed and strict lists were removed from both lists. An experimentally consistent literature network was generated from this remaining strict consensus sign genes using Metacore (Clarivate Analytics), using the following options: Build Network --> Shortest paths, maximum number of steps in path = 2, use canonical pathways, do not show disconnected seed nodes, and do not show shortest path edges only. Additionally, Pre-filters were selected to return only Transcription regulation, +P Phosphorylation, or -P Dephosphorylation edges known to exhibit either Activation or Inhibition effects. Nodes and edges were exported, then filtered locally to remove edges that were inconsistent with the experimentally observed direction of change from the relaxed consistent sign list. Additional nodes and edges were then removed for visualization purposes, in order to render a less tangled two-dimensional network visualized in Cytoscape.

**Cell Viability Assay**

Cells were plated at 2000-4000 cells per well in a black walled, clear bottom 96-well plate (Corning). After 24 hours, drugs were added at the desired concentrations and incubated another 72 hours. Viability of cells was measured with CellTiter-Glo Luminescent Cell Viability Assay reagent (Promega) as per manufacturer’s protocol and data were analyzed and visualized using GraphPad Prism.

**Crystal Violet Assay**

Cells were plated at 1000 cells per well in a 6-well plate. After 24 hours, cells were treated as indicated and then grown for an additional 9 days. Cells were then washed and fixed with cold methanol at -20°C and stained with 0.1% crystal violet solution. Stained plates were then washed, dried and imaged on a benchtop scanner.

**Scratch wound cell migration and invasion assays**

Scratch wound cell migration and invasion assays were carried out on the IncuCyte system (Essen Bioscience). For migration assays, cells were plated at ~25,000 cells /100 µl/well on to 96-well ImageLock plates (Essen Bioscience). The next day, 96-well WoundMaker (Essen Bioscience) was used to simultaneously create wounds in all wells. The plates were then gently washed to remove detached cells and desired treatments were added. For invasion assays, cells were plated at ~25,000-30,000 cells /100 µl/well on to 96-well ImageLock plates that have been pre-coated with 100 µg/ml of Matrigel (BD 354234). After allowing the cells to settle for 4 hours, scratches were made in the confluent cell monolayer using the WoundMaker. After washing plate to remove detached cells, wells were overlayed with 8 mg/ml Matrigel containing the desired drug treatments and allowed to polymerize for 30 minutes. An additional 100 µl of culture media with desired drug treatments were then added to each well of the plate. For both migration and invasion assays, plates were set up to be imaged in the IncuCyte system every 3 hours for 48-72 hours and images were acquired and visualized using the IncuCyte Zoom software. Further analyses were performed in GraphPad Prism.

**Activity-Based Protein Profiling (ABPP) sample preparation**

Cell lysates were prepared, and the ATP-binding proteome labeled according to the Pierce Kinase Enrichment Kits and ActivXTM Probes (Thermo Scientific, Rockford, IL, USA). Briefly, cell pellets were resuspended in 1ml of Pierce IP lysis buffer containing protease and phosphatase inhibitors (1:100) and sonicated. The lysates were cleared by centrifugation at 16,000g for 10 minutes at 4°C and desalted by Zeba Spin Desalting Columns (Thermo Scientific, Rockford, IL, USA). Protein concentration was measured using a Bradford assay, and a total of 1.5 mg per biological replicate was used for ATP probe labeling. MnCl2 was added to the lysate to a final concentration of 20 μM and incubated for 10 minutes. Lysates were incubated with drugs and then the desthiobiotin-ATP probe was added for the competition. All reactions were performed at room temperature. Reduction and alkylation were performed as recommended by the manufacturer. The lysates were then digested with 20 μg/mL of trypsin at 37°C overnight. A second digestion with 5 μg of trypsin was performed the next day for 1 hour. Desthiobiotinylated peptides were captured by 50 μl slurry of high-capacity streptavidin beads for 1h. The beads were washed with IP lysis buffer, PBS buffer, and LC-MS grade water in sequence with three washes for each step. The peptides were then eluted with elution buffer (aqueous 50% ACN, 0.1% trifluoroacetic acid (TFA)), lyophilized in a vacuum concentrator and re-suspended in 12 μl of injection buffer containing aqueous 2% acetonitrile, 0.1% formic acid and PRTC in order to confirm consistent operation of the LC-MS instrument. LC-MS/MS data were acquired as described above with the following changes. Sixteen tandem mass spectra were collected in a data-dependent manner following each survey scan, and desthiobiotinylation of lysine was set as a variable modification for database searching. MaxQuant data were normalized, log2 transformed, averaged and analyzed as described above in the phosphoproteomics methods. Differential expression between conditions was determined using 1.5-fold and p < 0.05 cutoffs.

**DNA/RNA sequencing and data analysis**

For RNA sequencing, extracted RNA was first screened for quality on an Agilent BioAnalyzer (Agilent Technologies, Inc., Wilmington DE). RNA-sequencing libraries were then prepared using the NuGen FFPE RNA-Seq Multiplex System (Tecan US, Inc., Morrisville, NC) wherein 100 ng of DNase treated RNA was used to generate cDNA and a strand-specific library following the manufacturer’s protocol. Library molecules containing ribosomal RNA sequences were depleted using the NuGen AnyDeplete probe-based enzymatic process. The final libraries were assessed for quality on the Agilent TapeStation, and quantitative RT-PCR for library quantification was performed using the Kapa Library Quantification Kit (Roche Sequencing, Pleasanton, CA). The libraries were sequenced on the Illumina NextSeq 500 v2 sequencer with a 75-base paired-end run in order to generate 90-100 million read pairs per sample. Extracted genomic DNA was screened with the Agilent TapeStation. For whole-exome sequencing using the Agilent SureSelect XT Clinical Research Exome kit (Agilent Technologies, Inc., Wilmington DE), 200 ng of DNA was used as input to construct libraries following the manufacturer’s protocol. Libraries were evaluated using the Agilent BioAnalyzer, and equimolar amounts of library DNA were used for whole-exome enrichment using the Agilent capture baits. The final libraries were assessed for quality on the Agilent BioAnalyzer, and quantitative PCR for library quantification was performed using the Kapa Library Quantification Kit. The libraries were sequenced on the Illumina NextSeq 500 v2 sequencer with two 75-base paired-end runs in order to generate 100-120 million read pairs per sample aiming for 100X target coverage.

For whole exome sequencing data, read adapters were detected using BBMerge (v37.88) (8) and subsequently removed with Cutadapt (v1.8.1). Processed raw DNA reads were then aligned to human genome HG19 using Burrows-Wheeler Aligner (BWA v0.7.7) (9). These aligned reads are processed with GATK (v2.2) (10) for base quality score recalibration, indel realignment, duplicate removal, and SNP and INDEL discovery and genotyping. Genomic variants are then annotated with ANNOVAR (11). Processed raw RNA reads were also aligned to human genome HG19 using STAR (v2.5.3a) (12). Gene expression was evaluated as read count at gene level with HTSeq (v0.6.1) and Gencode gene model v19, normalized and differential expression between experimental groups were evaluated. For analysis of the RNA sequencing data, genes were filtered using the filterByExpr function from the R edgeR package, resulting in 22,583 genes for downstream analysis (13). To account for differences in library size between the samples, trimmed mean of M values (TMM) normalization was applied, followed by data transformation using R voom package (14,15). Filtered and normalized genes were subjected to single sample gene set enrichment analysis (ssGSEA) using the cancer hallmarks (16).

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